

AMENDED CLAIMS **AP20 Rec'd PCT/PTO 17 MAY 2006**
received by the International Bureau on 29 June 2005 (29.06.2005): original claims 1-17 have been replaced by
amended claims 1-15 (4 pages).

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Statement

**THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY
OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:**

1. A method for detecting the presence or absence of a variant nucleotide in at least two SNP sites associated with thrombosis, said SNP sites selected from the group consisting of Factor V Leiden G1691A, Prothrombin (Factor II) G20210A, MTHFR C677T, MTHFR A1298C, Factor XIII G4377T, and tissue factor plasma inhibitor (TFPI) C536T, the method comprising the steps of;

a) amplifying regions of DNA containing the at least two SNP sites to form amplified DNA products;

b) hybridizing at least two tagged allele specific extension primers to a complementary target sequence in the amplified DNA products, wherein each tagged allele specific extension primer has a 3'-end hybridizing portion capable of hybridizing to the amplified DNA, and wherein the 3' end hybridizing portion of the at least two tagged allele specific extension primers comprise a sequence selected from the group consisting of bases 25 and up of SEQ ID NO: 1 to SEQ ID NO: 12, and a 5'-end tag portion complementary to a corresponding probe sequence, the terminal nucleotide of the 3' end hybridizing portion being either complementary to a suspected variant nucleotide or to the corresponding wild type nucleotide of the SNP site;

c) extending the at least two tagged allele specific extension primers, using labelled nucleotides, if the terminal nucleotide of the 3' end hybridizing portion is a perfect match to an allele of one of the SNP sites in the amplified DNA products;

d) hybridizing the at least two tagged allele specific extension primers to the corresponding probe sequence and detecting the presence of labelled extension products.

2. The method of claim 2 wherein the 5'-end tag portions of the at least two tagged allele specific primers comprises a sequence selected from the group consisting of bases 1 to 24 of SEQ ID NO: 1 to SEQ ID NO: 12.

3. The method of claim 1 wherein the probe sequence is coupled to a solid support.

4. The method of claim 3 wherein the solid support is selected from the group consisting of beads, spectrally coded beads, and a chip based microarray.

5. The method of claim 1 wherein the step of amplifying is conducted using a set of PCR amplification primers, said set comprising at least two pairs of PCR primers selected from the group of pairs consisting of:

SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, and SEQ ID NO: 23 and SEQ ID NO: 24.

6. A method for detecting the presence or absence of a variant nucleotide in at least two SNP sites associated with thrombosis, said SNP sites selected from the group consisting of Factor V Leiden G1691A, Prothrombin (Factor II) G20210A, MTHFR C677T, MTHFR A1298C, Factor XIII G4377T, and tissue factor plasma inhibitor (TFPI) C536T, the method comprising the steps of;

a) amplifying regions of DNA containing the at least two SNP sites to form amplified DNA products;

b) hybridizing at least two tagged allele specific extension primers to a complementary target sequence in the amplified DNA products, wherein the at least two tagged allele-specific extension primers are selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 12, each tagged allele specific extension primer having a 3'-end hybridizing portion capable of hybridizing to the amplified DNA, and a 5'-end tag portion complementary to a corresponding probe sequence, the terminal nucleotide of the 3' end hybridizing portion being either complementary to a suspected variant nucleotide or to the corresponding wild type nucleotide of the SNP site;

c) extending the at least two tagged allele specific extension primers, using labelled nucleotides, if the terminal nucleotide of the 3' end hybridizing portion is a perfect match to an allele of one of the SNP sites in the amplified DNA products;

d) hybridizing the at least two tagged allele specific extension primers to the corresponding probe sequence and detecting the presence of labelled extension products.

7. The method of claim 6 wherein the probe sequence is coupled to a solid support.
8. The method of claim 7 wherein the solid support is selected from the group consisting of beads, spectrally coded beads, and a chip based microarray.
9. The method of claim 6 wherein the step of amplifying is conducted using a set of PCR amplification primers, said set comprising at least two pairs of PCR primers selected from the group of pairs consisting of:
SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, and SEQ ID NO: 23 and SEQ ID NO: 24.
10. A kit for use in detecting the presence or absence of a variant nucleotide in at least two SNP sites associated with thrombosis, said SNP sites selected from the group consisting of Factor V Leiden G1691A, Prothrombin (Factor II) G20210A, MTHFR C677T, MTHFR A1298C, Factor XIII G4377T, and tissue factor plasma inhibitor (TFPI) C536T, said kit comprising a set of at least two tagged allele specific extension primers wherein each tagged allele specific extension primer has a 3'-end hybridizing portion including a 3' terminal nucleotide being either complementary to a suspected variant nucleotide or to the corresponding wild type nucleotide of one of the SNP sites and a 5'-end tag portion complementary to a corresponding probe sequence, and wherein the at least two tagged allele-specific extension primers are selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 12.
11. The kit of claim 10 further comprising a set of PCR amplification primers for amplifying regions of DNA containing the at least two SNP sites, said set comprising at least two pairs of PCR primers selected from the group of pairs consisting of:
SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, and SEQ ID NO: 23 and SEQ ID NO: 24.
12. The kit of claim 10 further comprising a set of probes.

13. The kit of claim 12 wherein the set of probes are coupled to a support.
14. A kit for use in detecting the presence or absence of a variant nucleotide in at least two SNP sites associated with thrombosis, said SNP sites selected from the group consisting of Factor V Leiden G1691A, Prothrombin (Factor II) G20210A, MTHFR C677T, MTHFR A1298C, Factor XIII G4377T, and tissue factor plasma inhibitor (TFPI) C536T, said kit comprising a set of PCR amplification primers for amplifying regions of DNA containing the at least two SNP sites, said set comprising at least two pairs of PCR primers selected from the group of pairs consisting of:
SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, and SEQ ID NO: 23 and SEQ ID NO: 24.
15. The kit of claim 14 further comprising a set of at least two tagged allele specific extension primers wherein each tagged allele specific extension primer has a 3'-end hybridizing